Lipid Metabolism Greases the Stem Cell Engine

Clifford D.L. Folmes,[1] Sungjo Park,[1] and Andre Terzic[1,*]
[1]Center for Regenerative Medicine, Mayo Clinic, Rochester, MN 55901, USA
*Correspondence: terzic.andre@mayo.edu
http://dx.doi.org/10.1016/j.cmet.2013.01.010

Metabolic plasticity is increasingly postulated to be vital in the transition between stemness maintenance and lineage specification. Knobloch et al. (2012) now demonstrate that regulation of lipogenesis by fatty acid synthase and Spot14-dependent malonyl-CoA supply determines the proliferative activity of resident neural stem cells, contributing to adult neurogenesis.

Energy generation is fundamental in the upkeep of cellular homeostasis, with fluidity in metabolic pathway utilization matching evolving and cell-specific bioenergetic demands. To ensure progenitor pool renewal and tissue rejuvenation, stem cells must maintain an exquisite balance between anabolism to create biomass, and catabolism to generate ATP as they transit from quiescence to active proliferation. Monitoring the dynamics of stem cell metabolism has pinpointed glycolysis in sustaining stemness, and oxidative metabolism in enabling lineage differentiation (Folmes et al., 2012). Glycolysis provides a compromise between concomitant ATP production and anabolic precursor supply, essential for stem cell self-renewal, while oxidative metabolism generates energy more efficiently to support the demanding functions of specialized progeny. Beyond fueling cellular needs, intermediary metabolism interacts with regulators of stem cell fate through nutrient-responsive metabolites and epigenetic regulation (Shyh-Chang et al., 2012), indicating that energy metabolism may prime cells to undergo fate conversions (Folmes et al., 2011). Indeed, modulation of stem cell metabolism is essential for organogenesis, with glycolysis promoting proliferation and survival of progenitors, while tissue differentiation requires a switch to more energetically efficient mitochondrial oxidative metabolism (Agathocleous et al., 2012; Folmes et al., 2012; Hom et al., 2011). Moreover, in distinct organ systems, caloric restriction appears to promote stem cell function, which can be attributed to regulation of a pivotal energy sensor (mTORC1) and modulation of energy metabolism (Cerletti et al., 2012; Yilmaz et al., 2012). A study published in *Nature* extends the emergent metabolic map of stem cell identity and shows that lipid biosynthesis underlies the proliferative capacity of stem and progenitor cells in the adult brain (Knobloch et al., 2012).

Lipogenesis is dependent upon the rate-limiting enzyme for fatty acid synthesis, fatty acid synthase (FAS), as well as on the availability of its precursor malonyl-CoA that relies on acetyl-CoA carboxylase (ACC) for synthesis and malonyl-CoA decarboxylase for degradation (Figure 1). Knobloch and colleagues demonstrate a preferential distribution of FAS in major areas of neurogenesis within the adult murine brain, with high expression in proliferating neural stem/progenitor cells (NSPCs) and reduced expression in differentiated progeny and nonmyelinating populations. Inhibition or deletion of FAS reduces proliferation of NSPCs, while conditional knockout of FAS—specifically in adult NSPCs—leads to the loss of lineage-traced NSPCs in neurogenic zones and a decline of newly formed neurons (Knobloch et al., 2012). Accordingly, FAS expression endows NSPCs with a distinctive metabotype in support of anabolic lipogenesis, enabling generation of lipid membranes required to sustain high stem cell proliferation.

Complementary modulators of de novo lipid synthesis, Spot14/thyroid hormone-responsive protein and Mig12 (Mid1-interacting protein), were also found to be vital for neurogenic NSPCs (Knobloch et al., 2012). Mig12 is an established activator of ACC, while Spot14 buffers this stimulatory role through dimerization with Mig12, resulting in reduced ACC activity, malonyl-CoA synthesis, and lipogenesis. Knobloch and colleagues report that isolated Spot14$^+$ cells proliferate slower than their Spot14$^-$ counterparts, with Spot14 knockdown favoring a proliferative nonradial NSPC state while Mig12 knockdown imposes slower proliferation. Overexpression of Spot14 alters the NSPC metabolic profile, consistent with reduced malonyl-CoA levels and impaired lipogenesis from acetate and glucose. The diminished anabolic potential induces a slower proliferating state, which is rescued by overexpression of Mig12. A Spot14-mediated impairment of lipid biosynthesis thus engages a metabolism-dependent brake on neurogenesis, through inhibition of cell proliferation and maintenance of neural stem cell quiescence (Figure 1).

The paradigm of lipogenesis-dependent regulation of neural stem cell fate provides the foundation for new avenues of investigation. For example, is the lipogenic control of phenotypes generalizable across species and tissues? What are the molecular underpinnings of lipogenesis-reliant stem cell function and destiny? Growing evidence indicates that the balance between anabolic and catabolic pathways regulates stem cell programming and reprogramming (Folmes et al., 2011, 2012). Beyond contributing to the anabolic phenotype of an actively proliferating cell, lipogenesis may impact lipid-sensitive signaling networks and transcription circuits. In this context, the ACC/MCD/malonyl-CoA axis may serve as a metabolic hub integrating the output of multiple signaling pathways, including those dependent on thyroid hormone and the energy sensor AMP-activated protein kinase, to modify stem cell behavior in response to fluctuations in the metabolic milieu within respective niches. Future studies are required to address if lipogenesis is dysregulated with age or disease, and whether lipid biosynthesis offers a targetable pathway to enable tissue regeneration.

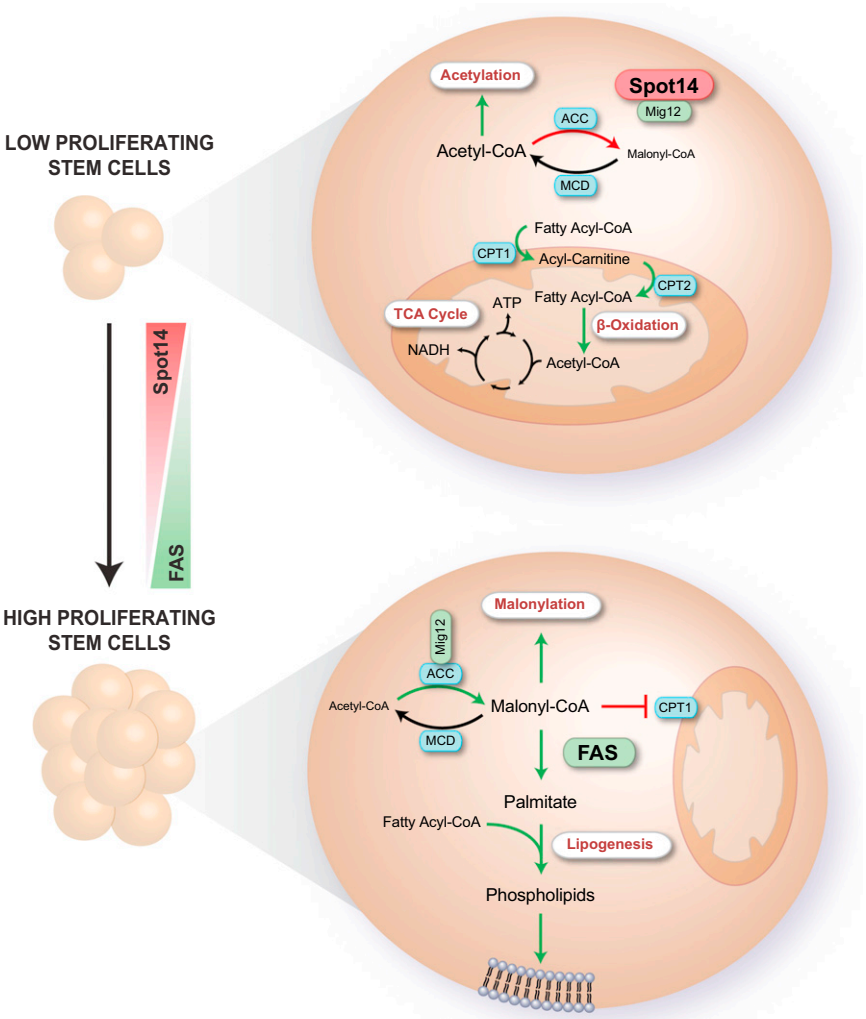

Figure 1. Stem Cell Proliferation Is Regulated by Fatty Acid Metabolism

In quiescent and low-proliferating stem cells, Spot14 has emerged as a metabolic brake on proliferation by binding and buffering the ability of Mid1-interacting protein (Mig12) to activate acetyl-CoA carboxylase (ACC), thus reducing the carboxylation of acetyl-CoA to malonyl-CoA. With conserved malonyl-CoA decarboxylase (MCD) activity, which is responsible for decarboxylation of malonyl-CoA back to acetyl-CoA, this action blunts lipid synthesis by reducing the availability of malonyl-CoA, the starting substrate for lipogenesis. This promotes a parallel stimulation of long-chain fatty acid oxidation by relieving malonyl-CoA inhibition of carnitine palmitoyl transferase 1 (CPT1), the rate-limiting step for long-chain fatty acid transport into mitochondria (via the carnitine palmitoyl transferase system including CPT2, carnitine palmitoyl transferase 2) and subsequent β-oxidation. Promotion of fatty acid oxidation and tricarboxylic acid (TCA) cycle activity may be critical to support the expanding energetic demands of differentiated progeny. Reduced Spot14 expression and increased fatty acid synthase (FAS) expression redirect fatty acid metabolism toward lipogenesis in support of the anabolic requirements of proliferating stem cells, including phospholipid generation essential for membrane synthesis. Downregulation of Spot14 favors stimulation of ACC through interaction with Mig12 and promotion of malonyl-CoA synthesis. A rise in cytosolic malonyl-CoA levels would supply the substrate for FAS, which is upregulated in high-proliferation cells, but would also repartition fatty acids away from oxidation to lipid biosynthesis by inhibiting CPT1. Regulation of lipogenesis by FAS and through the Spot14/ACC/malonyl-CoA axis thus represents a discrete pathway by which energy metabolism impacts stem cell fate.

An intriguing hypothesis is that malonyl-CoA acts as a rheostat of stem cell fate by regulating the equilibrium between anabolic lipogenesis and catabolic long-chain fatty acid β-oxidation. Malonyl-CoA is an endogenous inhibitor of carnitine palmitoyl transferase 1 (CPT1), the rate-limiting enzyme for long-chain fatty acid transport into the mitochondria and subsequent β-oxidation. High levels of malonyl-CoA provide an abundant substrate for fatty acid biosynthesis but would also suppress long-chain fatty acid oxidation and shunt fatty acids toward generation of biomass and lipid membranes, fundamental precursors for proliferating cells. Malonyl-CoA also contributes to lysine malonylation of metabolic enzymes and histones, which is under regulation of the NAD-dependent deacetylase sirtuin-5 (Du et al., 2011). This posttranslational modification may provide a yet-unidentified mechanism by which Spot14 expression impacts stem cell function. In contrast, low malonyl-CoA levels would redirect fatty acids to β-oxidation, promoting generation of ATP, NADH, $FADH_2$, and acetyl-CoA. Beyond energy supply, this profile could contribute to stem cell homeostasis by regulating generation/scavenging of reactive oxygen species to minimize cell damage, and by providing acetyl-CoA, a required substrate for protein acetylation—including histone acetylation, an epigenetic determinant of stem cell fate. Coordinated regulation of lipid biosynthesis versus oxidation may be broadly applicable to stem cell biology. This notion is exemplified by the peroxisome proliferator-activated receptor (PPAR)-dependent regulation of fatty acid oxidation that determines renewal versus differentiation of stem cell populations (Ito et al., 2012). Specifically, activation of PPARδ supports hematopoietic stem cell function and maintenance through asymmetric cell division, while inhibition of fatty acid oxidation promotes symmetric cell division for initial expansion followed by exhaustion of the progenitor pool (Ito et al., 2012).

A lipogenic requirement for tissue-specific stem/progenitor cell identity and proliferative behavior has thus now been established (Knobloch et al., 2012), advancing the paradigm of metabolic cell fate control. FAS and malonyl-CoA dynamics offer a metabolic branchpoint whereby lipids and associated precursors can be partitioned between lipogenesis and oxidation to sustain anabolic/catabolic homeostasis. In this way, lipid metabolism emerges as a bioenergetic controller of self-renewal versus differentiation, ensuring lifelong neurogenesis. Metabolism may thus represent a valuable target to promote stem cell function and tissue regeneration.

REFERENCES

Agathocleous, M., Love, N.K., Randlett, O., Harris, J.J., Liu, J., Murray, A.J., and Harris, W.A. (2012). Nat. Cell Biol. *14*, 859–864.

Cerletti, M., Jang, Y.C., Finley, L.W., Haigis, M.C., and Wagers, A.J. (2012). Cell Stem Cell *10*, 515–519.

Du, J., Zhou, Y., Su, X., Yu, J.J., Khan, S., Jiang, H., Kim, J., Woo, J., Kim, J.H., Choi, B.H., et al. (2011). Science *334*, 806–809.

Folmes, C.D., Nelson, T.J., Martinez-Fernandez, A., Arrell, D.K., Lindor, J.Z., Dzeja, P.P., Ikeda, Y., Perez-Terzic, C., and Terzic, A. (2011). Cell Metab. *14*, 264–271.

Folmes, C.D., Dzeja, P.P., Nelson, T.J., and Terzic, A. (2012). Cell Stem Cell *11*, 596–606.

Hom, J.R., Quintanilla, R.A., Hoffman, D.L., de Mesy Bentley, K.L., Molkentin, J.D., Sheu, S.S., and Porter, G.A., Jr. (2011). Dev. Cell *21*, 469–478.

Ito, K., Carracedo, A., Weiss, D., Arai, F., Ala, U., Avigan, D.E., Schafer, Z.T., Evans, R.M., Suda, T., Lee, C.H., et al. (2012). Nat. Med. *18*, 1350–1358.

Knobloch, M., Braun, S.M., Zurkirchen, L., von Schoultz, C., Zamboni, N., Arauzo-Bravo, M.J., Kovacs, W.J., Karalay, O., Suter, U., Machado, R.A., et al. (2012). Nature *493*, 226–230.

Shyh-Chang, N., Locasale, J.W., Lyssiotis, C.A., Zheng, Y., Teo, R.Y., Ratanasirintrawoot, S., Zhang, J., Onder, T., Unternaehrer, J.J., Zhu, H., et al. (2012). Science *339*, 222–226.

Yilmaz, O.H., Katajisto, P., Lamming, D.W., Gultekin, Y., Bauer-Rowe, K.E., Sengupta, S., Birsoy, K., Dursun, A., Yilmaz, V.O., Selig, M., et al. (2012). Nature *486*, 490–495.

How Sugar Tunes Your Clock

Gerald W. Hart[1,*]
[1]Department of Biological Chemistry, Johns Hopkins University, School of Medicine, 725 N. Wolfe Street, Baltimore, MD 21205-2185, USA
*Correspondence: gwhart@jhmi.edu
http://dx.doi.org/10.1016/j.cmet.2013.01.008

While cellular circadian clocks are set by the light/dark cycle, these clocks can be reset by what we eat. Two papers in this issue of *Cell Metabolism* reveal that *O*-GlcNAcylation of clock proteins, which is dependent on nutrients, adjusts our circadian clock (Kaasik et al., 2013; Li et al., 2013).

All of our cells have an ~24 hr clock (termed the circadian clock) that is primarily set by light but is also modified by environmental cues, especially nutrients, such as glucose (Damiola et al., 2000). However, the molecular mechanism of nutrient regulation of our cellular clocks is largely unknown. Current models suggest that our circadian clocks are comprised of transcriptional autoregulatory feedback loops controlled by specific transcription factors (Mohawk et al., 2012). Prior work has shown that the rate of cycling of the clock autofeedback loops are modulated by several regulatory mechanisms including post-translational modifications (PTMs) of clock transcription factors by acetylation, ADP-ribosylation, phosphorylation, and ubiquitination (Asher and Schibler, 2011). For example, in one such autofeedback loop, heterodimers of the transcription factors CLOCK and BMAL1 bind E-box elements in DNA to activate the expression of Period (Per) and Chryptochrome (Cry) proteins, which accumulate in the cytosol until they are phosphorylated and enter the nucleus where they inhibit the activity of CLOCK/BMAL1 (Figure 1). Degradation of the inhibitory Per and Cry proteins restarts a new cycle of the circadian clock (Ukai and Ueda, 2010). These transcription factor feedback loops are highly conserved in biology.

O-GlcNAcylation (Hart et al., 2011; Hart et al., 2007) is the covalent attachment and cycling of N-acetylglucosamine (GlcNAc) moieties on serine or threonine residues of nucleocytoplasmic proteins. Intracellular concentrations of UDP-GlcNAc, the donor for *O*-GlcNAcylation, are controlled by flux through several major metabolic pathways including metabolism of glucose, amino acids, and fatty acids. Because *O*-GlcNAcylation is highly responsive to UDP-GlcNAc concentrations, *O*-GlcNAc serves as a major metabolic/nutrient sensor (Hart et al., 2011). Prior work by Durgan et al. (2011) in mouse hearts showed that a diurnal cycle of *O*-GlcNAcylation is controlled by circadian clock regulation of glucose uptake, glutamine synthesis, and *O*-GlcNAc transferase (OGT) and *O*-GlcNAcase (OGA) protein levels, which add and remove *O*-GlcNAc from target substrates, respectively. They also showed that *O*-GlcNAcylation of the clock transcription factor Bmal1 reduced Per2 protein levels and phase-advanced the clock. These findings suggested that the circadian clock increases *O*-GlcNAcylation during the active/wake phase and that the sugar modification in turn modulates the timing of the circadian clock. Kim et al. (2012) further showed in *Drosophila* that *O*-GlcNAcylation of the Per transcription factor displays a circadian cycle, which tunes the clock by controlling Per nuclear entry and stabilization. Two papers published in this issue of *Cell Metabolism* (Kaasik et al., 2013; Li et al., 2013) have now substantially advanced our understanding of nutrient regulation of circadian clocks by showing that protein *O*-GlcNAcylation fine-tunes and adjusts our cellular clocks via the sugar's interplay with both phosphorylation and ubiquitination of clock regulatory proteins.

Using an unbiased phosphoproteomics approach, Kaasik et al. (2013) show that circadian cycling of phosphorylation regulates GSK3β kinase-dependent